Understanding the shrinking brain in multiple sclerosis: multimodal MRI studies

Steenwijk, M.D.

2016

document version
Publisher's PDF, also known as Version of record

Highlights

Quantification of MS pathology using high-spatial resolution MRI

- Representative, high quality and publicly available standard reference data sets should be developed to support the development of MS pathology quantification algorithms and facilitate the transfer of these methods to clinical practice.
- Adequate spatial resolution is an important aspect to consider when quantifying MS pathology, especially in the cortical gray matter.

The relationship between neurodegeneration and other pathology in MS

- Compared to relapsing-remitting MS, the weaker relationship between white matter pathology and cortical gray matter atrophy in progressive MS suggests a more independent neurodegenerative process.
- Distinct gray matter atrophy patterns potentially play an important role in the development of neurodegeneration and disease progression in MS
- Better models and longitudinal studies are required to further understand the spatiotemporal characteristics of gray matter atrophy in MS, the relationship with white matter pathology over time, and –even more important– the link with disability and cognitive deterioration.

The impact of neurodegeneration and other pathology on clinical disability in MS

- Although gray matter atrophy seems more closely related with cognitive impairment than with motor dysfunction, it is –due to their complex interrelationship– not easy to determine the most important imaging measure for explaining clinical impairment in MS.
- Future studies should determine the exact order in which different types of MS pathology (atrophy, normal-appearing tissue damage, focal lesions) occur, what their (causal) relationship is with respect to each other, and how this relates to disease progression and accumulation of clinical disability.

Chapter 5

Summary, general discussion and future perspectives

Summary and general discussion

The general aim of the studies presented in this thesis was to better understand the role of neurodegeneration in multiple sclerosis (MS), highlighting its relationship with other types of MS pathology and clinical impairment. Here we defined neurodegeneration as loss of gray matter (atrophy) that is visible magnetic resonance (MR) images of patients with MS. The thesis first focused on the segmentation and quantification of MS pathology: more sensitive and specific quantification of MS pathology can provide more reliable associations with measures of gray matter atrophy and can also be used to optimize quantification of gray matter atrophy itself. The subsequent section investigated the relationship between gray matter atrophy and other types of MS pathology in order to get a better understanding of the spatiotemporal development of neurodegeneration in MS. Finally, the clinical relevance of neurodegeneration was assessed by comparing the relationships of physical and cognitive scores with measures of gray matter atrophy and other types of MS pathology. This chapter will summarize the main findings with regard to the aims of this thesis defined in the introduction, being:

1. To improve the quantification of MS pathology on high-spatial resolution MR images to facilitate more accurate quantification of gray matter atrophy and allow for more reliable detection of relationships with other imaging and clinical measures.
2. To investigate the relationship between neurodegeneration and other pathology in MS.
3. To assess the impact of neurodegeneration and other types of pathology on clinical dysfunction in MS.

Moreover, for future research aiming to understand the role of neurodegeneration in MS, this chapter will present a number of opportunities which directly arise from our work.

Quantification of MS pathology using high-spatial resolution MRI

Since neurodegeneration in MS can be rather subtle, an important requirement for a better understanding of it is accurate quantification (1). Improved accuracy and sensitivity of imaging methods reduce the number of patients that is required to detect subtle associations or to measure treatment effects in trials. The desire for improved imaging methods is not limited to the quantification of gray matter atrophy alone, but also includes the quantification of other types of MS pathology such as focal lesions and normal-appearing tissue damage. Improved quantification of these imaging measures will provide more reliable associations with gray matter atrophy and can also be used to get better estimates of gray matter atrophy

itself. The reason for this is that most gray matter atrophy measurement methods are developed for healthy brains and cannot handle the (especially focal) pathology that is present in MS patients: they will assign voxels with visible MS pathology to incorrect tissue classes and thereby introduce bias in the gray matter atrophy measurements (2–5). Therefore, in Chapter 2 we aimed to improve two methods that quantify different aspects of other MS pathology (i.e., white matter lesion load and normal-appearing tissue damage). The improved methods were subsequently applied in the other studies described in this thesis.

Accurate automated white matter lesion segmentation and quantification

High-spatial resolution 3D MR images are recommended for segmentation and quantification of MS pathology (1). The high resolution reduces partial volume effects that may occur when transforming the resulting lesion masks to other time points or image contrasts (e.g., registration of the lesion mask to the T1-weighted image in order to reduce the interference of white matter lesions with gray matter atrophy quantification). However, since manual quantification of MS lesions is not feasible on high resolution images that typically contain more than 100 slices per subject, there is a need for reliable automated lesion segmentation techniques. We therefore developed and evaluated a method to automatically perform lesion segmentation in patients with MS in Chapter 2.1. Based on the literature (6,7), we chose to further develop the *k* nearest neighbor (*k*NN) technique by optimizing intensity normalization and adding so-called tissue type priors. Using a leave one out approach, we searched for the optimal configuration by comparing the volumes and spatial overlap with manually labeled reference segmentations in 20 patients with MS. The results of this study showed that intensity normalization had a large influence on the segmentation performance. Moreover, in line with the literature (8), the use of tissue type priors increased segmentation performance especially by reducing the rate of detection errors. Comparing the performance of our method to others was difficult due to the high dependency of performance metrics on the data set and reference segmentation used. Given this consideration, it was concluded that the developed *k*NN-TTP method outperformed the original *k*NN approach and performed at least equal to other automated white matter lesion segmentation approaches.

Detection of changes in the gray matter of MS patients using high-spatial resolution T1-relaxation time measurements

T1-relaxation time (T1-RT) mapping is a promising quantitative MRI technique for in vivo quantification of normal-appearing tissue damage in MS. Previous studies reported widespread changes in the gray matter of MS patients (9–12), but were limited by a relatively low trough-plane resolution which may have interfered with the measurements – especially due to partial

volume effects with cerebrospinal fluid (CSF). Chapter 2.2 therefore investigated whether high-spatial resolution T1-RT mapping can be used to detect changes in the normal-appearing gray matter of patients with MS. In contrast to the results of previous studies, no differences were found when comparing conventional normal-appearing gray matter (NAGM) T1-RT histogram parameters (i.e., mean, peak position, peak height, peak width) between MS patients and healthy controls. Only the more sophisticated 'skewness' measure (describing the asymmetry / tails of the histogram) revealed differences: in both the thalamus and cortex, the T1-RT histogram skewness was higher in patients compared to healthy controls. Moreover, increased cortical T1-RT skewness showed a strong association with cognitive impairment. In absence of conventional histogram parameter differences, increased T1-RT skewness reflects higher T1-RT in a subset of the voxels. This underlines that the cortical and thalamic tissue damage found in our study is rather subtle and localized (in contrast to widespread tissue damage that would not alter skewness but increase peak position). Given the consistency of our results with several recent post-mortem findings (13,14), the discrepancy between our results and the previous in-vivo studies was best explained by differences in the spatial resolution used for T1-RT map acquisition. Whereas our study used a near-isotropic high-spatial resolution, none of the former studies used a sequence with a slice thickness lower than 4 mm. It was concluded that such thick slices may lead to large partial volume effects, especially in the cortex, which may be even more pronounced in the presence of atrophy due to MS.

Discussion

The aim of Chapter 2 was to improve MS pathology quantification on high-spatial resolution MR images. To this end, we developed a method for automatic MS lesion quantification, and evaluated the ability of high-resolution T1-RT mapping to detect changes in NAGM of patients with MS.

Despite the labour intensive work and the publication of several automated methods, manual labelling is still the gold standard for white matter lesion segmentation in trials and MS imaging studies (15–18). Several explanations may account for this, including the fact that various automated methods may provide different results depending on the scanner, sequence and contrast of the image used. Moreover, it is hard to compare the performance of the individual methods as most studies used their own reference data sets, images and evaluation metrics to quantify segmentation performance. A possible solution for this problem is the development of a standard reference data set that is publicly available, representative for the general MS population, comprises data from multiple centers, field strengths and (clinical) sequences, and is outlined by multiple experienced raters. An attempt into this direction was made

during the MICCAI conference in 2008: here, imaging and reference data of 51 patients with MS from two centers were made available in order to facilitate an MS lesion segmentation challenge amongst the visitors of the MICCAI conference (http://www.ia.unc.edu/MSseg) (19). After the conference, the data set was made available to the community in order to evaluate the performance of new automated lesion segmentation methods and compare their performance. However, in practice, the usability of this data set appears to be limited: the imaging data is of rather low quality, double-rater manual reference segmentations are not available for all subjects, and the reference segmentations seem to be of limited quality (20). Moreover, the validation procedure in this challenge is fixed and only determines a limited number of performance metrics. Several widely accepted measures of volumetric agreement (e.g., intra-class correlation coefficient) (21) or spatial agreement (e.g., Dice's similarity index, detection error rate or outline error rate) (22–24) are not part of the evaluation scheme which limits the comparability of the outcome measures with other studies. Good quality data and flexible evaluation procedures are crucial for the successfulness of future standard reference data sets (and automated lesion segmentation methods in general).

The importance of applying adequate imaging techniques for individual research questions was emphasized by our study that investigated gray matter T1-RT at high-spatial resolution in patients with MS. While previous studies reported widespread T1-RT changes in the gray matter, our study could only detect subtle changes. The discrepancy can most likely be explained by the inadequate spatial resolution used in those previous studies, which examined the gray matter with slices much thicker than the cortex itself. This resulted in profound partial volume effects that were even more pronounced in patients with MS due to the presence of disease-related gray matter atrophy. Since the maximum feasible acquisition time often restricts the spatial resolution that can be clinically achieved, this finding may have significant consequences for the clinical applicability of quantitative MRI techniques in general – especially when imaging the cortical gray matter. Therefore, the suitability of (quantitative) MRI sequences for specific research questions should be carefully assessed before devising and acquiring new MS protocols. In addition, efforts should be made to explore faster acquisition of quantitative MRI data, possibly by exploiting the benefits of new hardware and higher magnetic field strengths. Meanwhile, new post-processing techniques accounting for partial volume effects should be developed to maximally exploit the potential of the currently available acquisition techniques.

The relationship between neurodegeneration and other pathology in multiple sclerosis

Gray matter atrophy is a crucial component of MS: it is present early, accelerates over time, and explains clinical (especially cognitive) impairment to a better extent than white matter pathology (25). Although neuroaxonal loss was recently reported as the pathological substrate of MRI-measured gray matter atrophy (26), it is unclear whether this is driven by primary gray matter damage or secondary to white matter pathology (27). From the inconclusive associations found by MRI-studies that investigated the presumed relationship between gray matter atrophy and white matter pathology (28–33), it is clear that the gray matter loss occurring in MS patients cannot be explained by white matter pathology alone. Moreover, the increasing atrophy rate during the course of the disease (34), suggests the presence of an independent primary neurodegenerative process. However, most studies in MS investigated patients with a relatively short disease duration, in which the independent neurodegenerative effect may be too small to be observable. Therefore, Chapter 3 aimed to investigate the relationship between gray matter atrophy and other types of MS pathology more comprehensively in a large cohort of patients of 208 patients with long-standing disease.

What explains gray matter atrophy in long-standing multiple sclerosis?

Chapter 3.1 sought for the whole-brain white matter pathology measures that statistically explained whole-brain, cortical and deep gray matter atrophy in patients with long-standing MS. Atrophy was present in all gray matter regions of interest, and while whole-brain and deep gray matter atrophy were mostly explained by smaller white matter volumes, cortical atrophy was best explained by reduced white matter integrity. In addition, higher lesion load, older age and male sex were associated with more gray matter atrophy. Investigating the relationship in clinical subtypes revealed remarkable differences: in patients with progressive MS, the relationship between gray matter atrophy and white matter pathology was much weaker than in those with relapsing-remitting (RR) MS. Consistent with the observation that gray matter atrophy cannot be fully explained by white matter pathology, the results of this study indicated that the neurodegenerative process in patients with progressive disease becomes more independent from white matter pathology.

Local gray matter atrophy and anatomically connected white matter pathology in multiple sclerosis

After investigating the relationship between gray matter atrophy and white matter pathology at the whole-brain level, the logical next step in Chapter 3.2 was to examine whether such

relationships also exist between regional gray matter atrophy and pathology in anatomically connected white matter tracts. In order to do so, the anatomically connected white matter tracts corresponding to several cortical and deep gray matter regions were derived using probabilistic tractography in healthy controls. After warping the resulting atlas to the patient scans, the amount of lesion volume and integrity of the tracts was quantified and used to investigate associations with atrophy in the corresponding gray matter regions. Similar to the results of the previous chapter, regional gray matter atrophy was explained by pathology in anatomically connected white matter tracts. While deep gray matter atrophy was mainly associated with lesion load in connected tracts, cortical atrophy was particularly associated with reduced integrity of the tracts (especially in the temporal regions). In addition, also corroborating the results of the previous chapter, the relationships between cortical gray matter atrophy and connected white matter pathology were much weaker in secondary-progressive (SP) MS patients than in patients with RRMS. This strengthened the idea that cortical gray matter atrophy and white matter pathology are (at least partly) independent disease processes in patients with progressive MS.

Patterns of gray matter atrophy in MS: their evidence, association with white matter pathology and clinical relevance

Given the notion of changing relationships between gray matter atrophy and white matter pathology in different clinical subtypes, we realized that in MS little is known about the spatiotemporal development of gray matter atrophy itself. Therefore, a different approach was adopted in **Chapter 3.3**, which investigated whether gray matter atrophy in patients with long-standing MS is largely a diffuse 'global' process or, instead, occurs in distinct anatomical patterns. For this purpose, vertex-wise cortical thickness maps were analysed with source-based morphometry. This technique allows to decompose the gray matter atrophy visible on MRI into specific patterns. The weight of a pattern in a specific subject is represented by the so-called loading factor, which allows to identify clinically relevant patterns by group-wise comparison. Interestingly, six of the ten cortical thickness patterns had significantly lower loadings in patients with MS compared to healthy controls. The largest group-wise differences were found in the pattern predominantly involving the bilateral temporal pole and the pattern involving the bilateral posterior cingulate cortex. In MS, the loadings of these specific patterns were negatively correlated with whole-brain lesion load (i.e., patients with higher lesion loads had smaller cortical thickness in the regions corresponding to these patterns), whereas the loadings of most other patterns were associated with reduced white matter integrity. Importantly, several patterns showed stronger associations with clinical impairment than global atrophy. Of special interest was atrophy of the posterior cingulate cortex, which

emerged as the pattern most closely related to cognitive impairment, and showed –in contrast with the findings in the previous chapters– stronger associations with white matter pathology in SPMS patients than in RRMS patients.

Discussion

The aim of Chapter 3 was to investigate the relationship between gray matter atrophy and other types of MS pathology more comprehensively in a large cohort of patients with long-standing disease. In line with MRI-studies that were previously performed in patients with a short disease duration (35–37), our studies revealed a clear relationship between gray matter atrophy and white matter pathology in the patients with (long-standing) relapsing-remitting MS. However, the situation was different in patients with progressive MS: in these patients a relationship between cortical atrophy and white matter pathology was lacking.

In line with the literature, our studies consistently identified (connected) lesion load as the white matter pathology measure that is most closely related to deep gray matter atrophy in MS (35,36). The stronger link between reduced normal-appearing white matter integrity and cortical atrophy has only been reported by one study before, which investigated the relationship between white matter pathology in the corticospinal tract and sensorimotor cortex atrophy in a smal cohort of RRMS patients (38). At present it is unclear why deep and cortical gray matter atrophy have different associations with white matter pathology in MS. It could be that other types of MS pathology, such as cortical lesions, play a role: although histopathological and imaging studies found a limited impact of (juxta)cortical lesions on gray matter atrophy (39,40), imaging studies have suggested a relationship between cortical lesion load and diffuse white matter damage (41). Alternatively, the underlying anatomical structure of the brain might play a role: while most MS lesions (i.e., 'focal' demyelination) project onto the deep gray matter structures –and thereby cause deep gray matter atrophy–, the effect of MS lesions on the cortical gray matter is much more widespread, resulting in a relatively larger impact of normal-appearing white matter damage (e.g., 'subtle' demyelination) on the cortex. Therefore, future studies should come up with better models to investigate the effect of white matter pathology on gray matter atrophy. They should examine a more complete spectrum of MS pathology measures and should incorporate regional differences in structural connectivity (i.e., many tracts project onto central structures like the thalamus while cortical regions have much more specific connections). Moreover, longitudinal studies should be performed to better understand the (changing) relationships between white matter pathology and gray matter atrophy during the course of the disease.

The weaker relationship between white matter pathology and cortical gray matter atrophy in

progressive patients has, according to our knowledge, not been reported before. The finding is however consistent with the observation that the (accelerating) amount of gray matter atrophy in MS cannot be explained by white matter lesions alone. Searching for an explanation we hypothesized that the neurodegenerative process in progressive MS is more independent from white matter pathology in progressive MS compared with RRMS. This shifted our focus from the gray matter atrophy / white matter pathology relationship to gray matter atrophy itself. The detection of various distinct clinically relevant cortical atrophy patterns provided important new insights into the spatiotemporal characteristics of neurodegeneration in MS. It was realized that specific clinical symptoms may be better explained by specific atrophy patterns than by global atrophy. And differences in clinical subgroups suggested that atrophy patterns could be crucial to understand the more independent neurodegenerative process in patients with progressive MS compared to RRMS. Especially the posterior cingulate cortex appeared of interest: atrophy in this region was more pronounced in SPMS than in RRMS, showed stronger associations in SPMS compared to RRMS, and was the best explanatory variable for cognitive impairment. Although atrophy of the posterior cingulate cortex has been previously described in MS (42,43), our results suggested that the importance of atrophy in this area has not yet been fully recognized in the literature. The posterior cingulate cortex is one of the most important hubs in the human brain (44), is affected in various neurodegenerative diseases (45), and has been linked to network collapse and cognitive impairment in various functional imaging studies of MS patients (46). The fact that our work, aiming to investigate atrophy patterns instead of brain function, led to similar results, underlines the need for a better understanding of the (possibly crucial) role of this area.

The studies in this chapter do not explain why neurodegeneration is a more independent process in progressive MS, and neither why cortical atrophy would follow a non-random pattern in MS. Based on our results, we hypothesize that in patients with *early MS*, gray matter atrophy develops largely secondary to white matter pathology. This initial gray matter atrophy effect is rather limited and associated with white matter pathology, but may lead to a 'second-order effect' that indirectly causes atrophy in other connected areas. In this model, densely connected brain hubs (e.g., the posterior cingulate cortex and thalamus) might be more at risk of developing atrophy, because of their central position in the network. At a specific point in time (i.e., the *conversion-point*), atrophy in these central structures has accumulated such that they start to fail. This leads to widespread communication failure in the brain, atrophy that may be (at the whole-brain level) largely unrelated to white matter pathology, and also (accelerated) disease progression (see Figure 1). The truth value of this hypothesis needs to be evaluated in future work. Future research should therefore investigate the spatiotemporal characteristics of gray matter atrophy in MS, the relationship with white matter pathology,

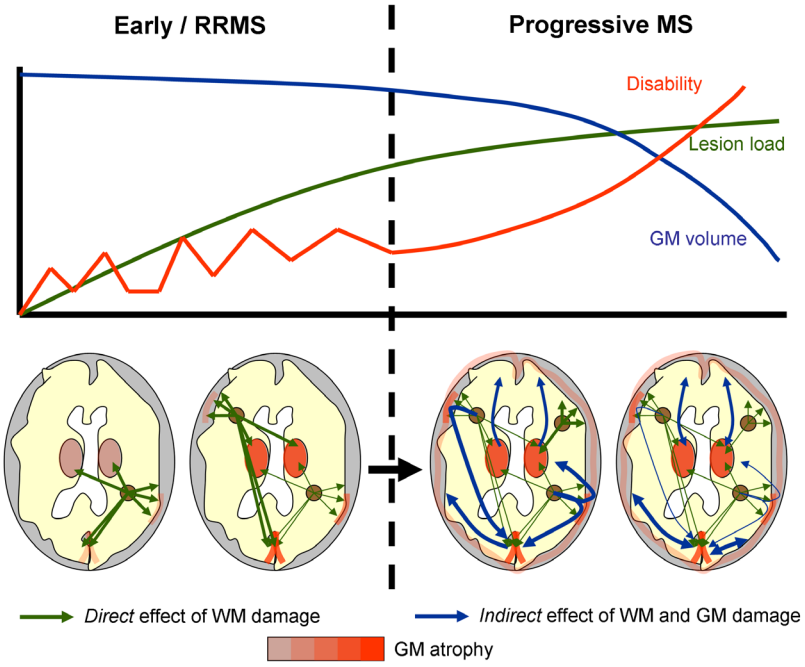

Figure 1. Figure illustrating the changing relationship between gray matter atrophy and white matter pathology during the disease course of MS. Early in the disease, MS lesions lead to atrophy in remote, *directly* connected, gray matter regions. Because of their position in the structural network, central gray matter regions are more at risk to develop atrophy, in contrast to the cortical gray matter regions, on which the effect of lesions is much more limited. At the so-called *conversion-point*, central structures have accumulated such damage that they start to fail. This will *indirectly* affect the functioning of regions throughout the brain, thereby inducing much more widespread GM damage and nonlinear acceleration of GM atrophy.

and –even more importantly– the link with measures of disability and cognitive deterioration. Specific research questions that could be addressed in such future studies are: 1) whether similar atrophy patterns are also present in the deep gray matter, and 2) whether the patterns can be used to predict future neurodegeneration, clinico-cognitive status and disease progression in MS.

The impact of neurodegeneration and other pathology on clinical disability in multiple sclerosis

Although gray matter atrophy explains clinical impairment to a greater extent than MS lesions (25), it is not exactly known whether this is primarily the result of 'diffuse' atrophy, tissue loss in specific regions, or a combination of several types of MS pathology. Therefore, Chapter 4 reassessed the clinical impact of gray matter atrophy compared to other types of pathology in MS.

The importance of whole-brain white matter integrity, lesions and gray matter volume for cognitive impairment in multiple sclerosis

In Chapter 4.1, we investigated the impact of white matter integrity, MS lesions and gray matter volume on cognitive impairment in a cohort of 35 cognitively preserved and 18 cognitively impaired MS patients (average disease duration: 11.6 and 11.9 years, respectively) that was imaged at 1.5 Tesla. While no differences were found in lesion and in atrophy measures, widespread changes were detected in the normal-appearing white matter of the patient groups. Moreover, compared to healthy controls, cognitively impaired patients showed more extensive fractional anisotropy reductions in the white matter than cognitively preserved patients. Although the areas with normal-appearing white matter damage partly overlapped in both cognitive phenotypes, the cognitively impaired patients showed additional damage in areas that were highly relevant for cognitive functioning (juxtacortical areas, thalamus and infratentorial white matter). A qualitative interpretation of the results of the voxel-based analysis revealed that reduced white matter integrity occurred largely independent of gray matter atrophy and lesion load. Taken together, this study indicated that subtle white matter damage may be an important imaging substrate of cognitive impairment in MS and that diffusion tensor imaging may be a powerful tool when monitoring cognitive impairment in MS.

The relevance of damage in thalamic tracts for cognitive and neuropsychiatric impairment in multiple sclerosis

The importance of the thalamus in cognitive impairment in MS has been highlighted by several previous studies (47–49). Following the results from the previous chapter, the important role of the thalamus in cognitive processes, and the fact that information processing speed is usually most early and severely affected in MS (50), we hypothesized that subtle damage in the thalamocortical connections may be more responsible for cognitive decline in MS than was thought previously. Therefore Chapter 4.2 investigated the impact of damage in

different afferent pathways of the thalamus on cognitive and neuropsychiatric impairment in patients with MS through an international collaboration. Seventy-three patients with MS (average disease duration: 11.5 years) and 18 healthy controls were examined with extensive neuropsychological evaluation and MRI at 3 Tesla. The individual thalamic nuclei (i.e., anterior, posterior, lateral, medial) were segmented using an histopathological atlas (51) and tractography was used to quantify the lesion load and integrity of the connected thalamic tracts. The results of this study showed that lesion load and normal-appearing white matter damage in especially the anterior and posterior thalamic tracts was associated with cognitive impairment in patients with MS. Disinhibition and agitation were most closely related to thalamic tract integrity and volume respectively. Altogether, this study showed that changes within thalamic tracts play an important role in understanding cognitive impairment and disinhibited behaviour in patients with MS.

Understanding motor dysfunction in long-standing multiple sclerosis

After investigating the clinical relevance of neurodegeneration in MS cohorts with a relatively short disease duration, a logical next step was to investigate the cohort of 208 patients with long-standing MS. In these patients, differences in pathological (especially neurodegenerative) measures and physical decline are expected to be more pronounced compared to patients with a shorter disease duration (52). **Chapter 4.3** started by unravelling the neuroimaging substrate of motor dysfunction in long-standing MS. In all subjects, the corticospinal tracts were segmented using an atlas that was obtained with tractography in the healthy controls. Atrophy of the cortex connected to these tracts, lesion load within the tracts and measures of normal-appearing white matter integrity within the tracts were determined. In addition, the analysis included measures of whole-brain atrophy, spinal cord pathology and cerebellar pathology to investigate a wide spectrum of imaging markers that may play a role. The results of this study showed that infratentorial and spinal cord damage were most closely related to various measures of motor functioning. In addition, atrophy of the cortex connected to the corticospinal tract appeared to play an important role. It was concluded that motor dysfunction in MS has a complex substrate and cannot be ascribed to a single neuroimaging measure.

The neuroimaging substrate of cognitive impairment in long-standing multiple sclerosis

Chapter 4.4 focused at the neuroimaging substrate of cognitive dysfunction in long-standing MS. Although an increasing amount of literature is available on specific imaging measures and/or brain regions in relation to cognitive impairment in MS (53–57), only a few studies combined these approaches aiming to discern the most important imaging measures

(58,59). Therefore, this chapter took a more integrative approach, aiming to identify the neuroimaging measures most closely related to cognitive impairment from a wide spectrum of MS pathology measures. The results of this study showed that, out of all imaging measures, deep gray matter atrophy and reduced white matter integrity are the strongest statistical predictors for cognitive impairment. The associations between the measures of MS pathology and cognitive performance were approximately twice as strong in cognitively impaired patients compared to cognitively preserved patients. It was concluded that in long-standing MS the same cognitive domains are affected as in early MS, but more pronounced. Moreover, the study showed that deep gray matter atrophy and reduced white matter integrity are the most important neuroimaging predictors for cognitive deterioration in MS.

Discussion

The aim of **Chapter 4** was to assess the impact of gray matter atrophy and other types of pathology on clinical dysfunction in MS. Different from previous studies investigating a limited number of neuroimaging measures or brain regions, we investigated a wide spectrum of measures in multiple patient cohorts to identify the imaging substrate of each of the clinical outcome measures. Despite the fact that none of the clinical measures could be explained by a single neuroimaging marker, atrophy measures recurrently evolved as a strong predictor for clinical impairment.

In line with the literature, deep gray matter atrophy –driven by volume loss of the thalamus– showed consistently strong associations with *cognitive impairment* (48,55,60,61). Also in line with previous findings, reduced white matter integrity evolved as an independent predictor for worse cognitive performance (57). More specifically, compared with cognitively preserved patients, cognitively impaired patients showed more prominent white matter integrity reductions in regions that are important for cognition. Similar cognitive domains were affected in patients with long-standing MS as in patients with early MS. Cognitively impaired patients showed two times stronger correlations of the imaging measures with cognitive performance than cognitively preserved patients. The fact that both deep gray matter atrophy and reduced white matter integrity evolved as predictors for cognitive impairment is confusing to some extent: in **Chapters 3.1** and **3.2**, we found that deep gray matter atrophy is associated with lesion load and reduced white matter integrity is associated with cortical thinning. This could suggest that the other types of MS pathology (i.e., lesion load and cortical atrophy) also play a role in cognitive impairment. However, the previous chapters did not find a clear relationship between deep gray matter atrophy and reduced white matter integrity themselves. This emphasizes the fact that collinearity of the evaluated neuroimaging measures may have had substantial impact on the predictors that were finally selected in the statistical models.

Apparently, the large number of neuroimaging measures limits the value of the individual measures, given the fact that they are hardly independent. This sets the question whether it is really helpful to know the 'strongest' imaging predictors of cognitive dysfunction. It could be that it is much more valuable to investigate the spatiotemporal characteristics of brain changes in MS and its (co-occuring or 'lagging') relationship with the accumulation of the cognitive problems.

Both neuroinflammatory and neurodegenerative markers were identified as explanatory variables of *physical disability*. In line with the literature (58,62), especially infratentorial and spinal cord pathology (in both cases lesions to a greater extent than atrophy) appeared to have predictive value. In addition, thinning of the cortex connected to the corticospinal tract explained upper limb disability and lesions in the corticospinal tract explained lower EDSS scores. The integrity of the corticospinal tract was not predictive for any of the measures, which could –again– be explained by collinearity of cortical atrophy with reduced white matter integrity and lesions as found in **Chapters 3.1** and **3.2**.

Based on the above, it is clear that it is hard to determine which imaging measure (gray matter atrophy or other types of MS pathology, either neurodegenerative or neuroinflammatory) is most helpful in explaining impairment in MS. Our results tend to suggest that gray matter atrophy in the brain is more closely related to cognitive deficits than physical disability, but this verdict is complicated by the complex interrelationship of the different measures, which is furter complicated by the spatial dependence and changing course during the disease. Combining all these results, it can be said that we might have been too naive, presuming that we would be able to separate all these measures and would be able to find individual measures explaining specific aspects of clinical dysfunction in MS. Therefore, the grand challenge for future studies is to determine the exact order in which different types of MS pathology (atrophy, normal-appearing tissue damage, focal lesions) occur, what their (causal) relationship is with respect to each other, and how this relates to disease progression and accumulation of clinical disability.

Conclusions

Quantification of MS pathology using high-spatial resolution MRI

- Accurate automated quantification of focal MS pathology on isotropic MR images is feasible.
- Adequate spatial resolution is an important aspect to consider when quantifying MS pathology, especially in the cortical gray matter.

The relationship between neurodegeneration and other pathology in MS

- Patients with progressive MS display a weaker relationship between gray matter atrophy and white matter pathology than patients with RRMS, which suggests a more independent neurodegenerative disease process in progressive patients.
- Gray matter areas central in the structural network of the brain might play an important role in the development of neurodegeneration and disease progression.

The impact of neurodegeneration and other pathology on clinical disability in MS

- Due to the high collinearity and complex interrelationship of MS pathology measures, it is hard to discern the imaging measures that best explain different aspects of clinical disability.
- Studies investigating clinical impairment in MS should examine the whole anatomical system (i.e., studies investigating physical impairment should incorporate cerebellar and spinal cord MS pathology), rather than only the brain.

Future perspectives

Quantification of MS pathology using high-spatial resolution MRI

- Representative, high quality and publicly available standard reference data sets should be developed to support the development of MS pathology quantification algorithms and facilitate the transfer of these methods to clinical practice.
- Combined histopathology and MRI studies at high-field strength are needed to further elucidate the pathological substrate of changes measured with quantitative MRI techniques.

The relationship between neurodegeneration and other pathology in MS

- More accurate models, incorporating regional information and the underlying structural network, should be constructed to better understand the close interrelationship between measures of gray matter atrophy and other MS pathology.
- Longitudinal studies are required to further understand the spatiotemporal characteristics of gray matter atrophy in MS, the relationship with white matter pathology and the relationship with disease progression in MS.

The impact of neurodegeneration and other pathology on clinical disability in MS

- Future studies should determine the exact order in which different types of MS pathology (atrophy, diffusivity changes, focal lesions) occur, what their (causal) relationship is with respect to each other, and how this relates to disease progression and accumulation of clinical impairment.

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